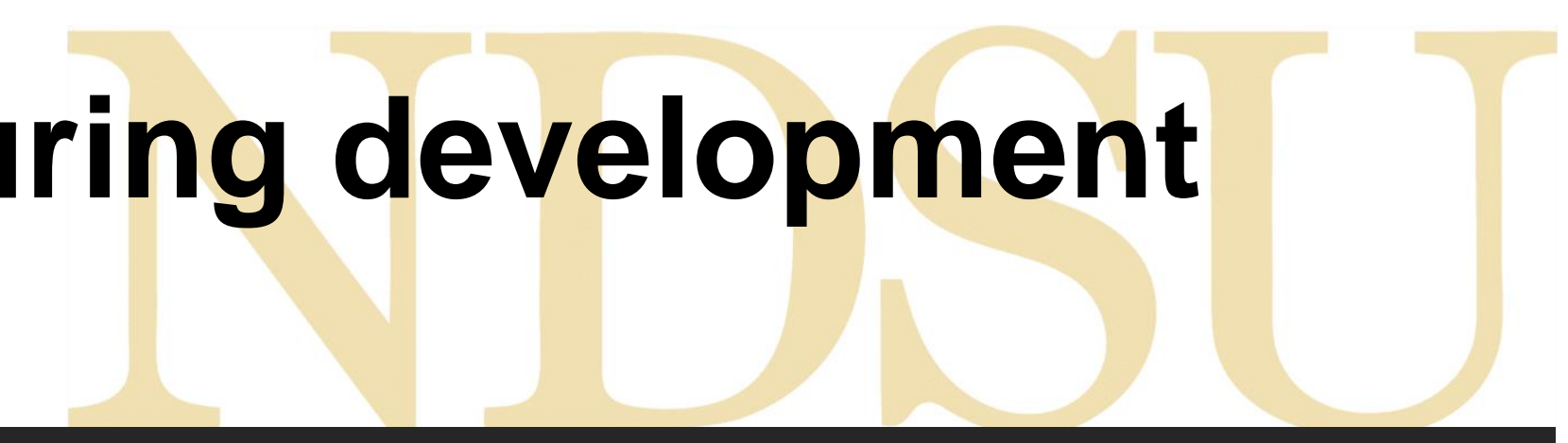


Hacking the solitary bee: connecting hormonal dynamics with underlying molecular mechanisms during development

Laurie M. Agosto^a, Bryan R. Helm^b, Jason Holthusen^c, Alex S. Torson^b, George D. Yocum^d, Kendra J. Greenlee^b, Julia H. Bowsher^b

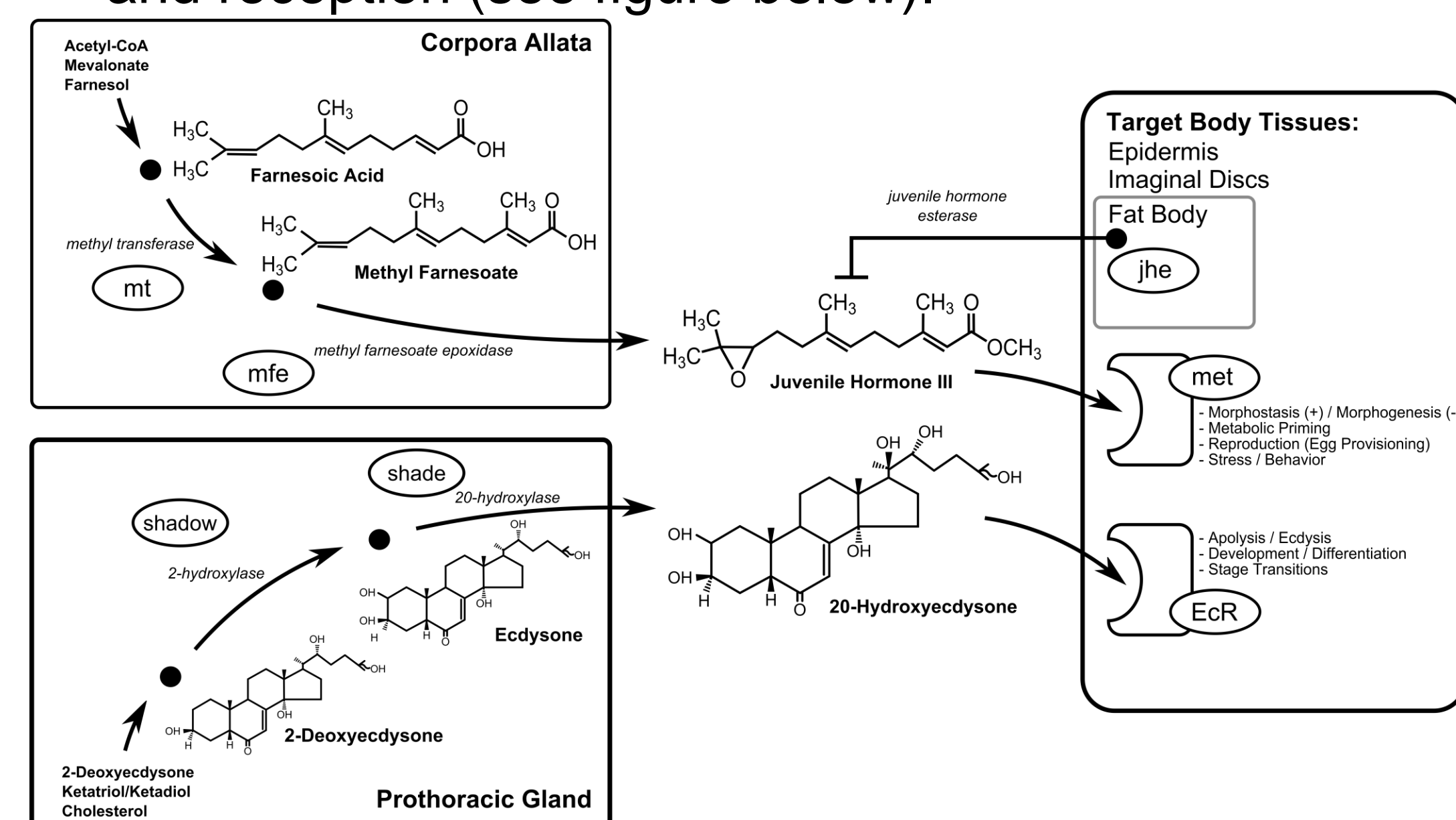


Abstract

Insects—especially holometabolous—undergo a complex metamorphosis in form and function from the immature to mature stage of their life cycle. Physiologically, metamorphosis is regulated by hormones, primarily juvenile hormone and ecdysone, which control different aspects of the metamorphic processes¹⁻³. However, much of our understanding of metamorphosis is based upon studies focusing on just a few model organisms, and connections between the physiological dynamics and their underlying molecular mechanisms remain poorly described¹⁻³. Here, we simultaneously characterize the developmental physiology and corresponding molecular mechanisms of larval to adult metamorphosis in the alfalfa leaf cutter bee, *Megachile rotundata*. We measured the hemolymph titer of juvenile hormone III (JHIII) using a recently established HPLC-MSMS protocol⁴. From these same individuals, we quantified the expression of genes that regulate JHIII synthesis, degradation, and reception in target tissues. While we did not directly assay ecdysone quantities in hemolymph for this study, we quantified expression of genes that regulate its synthesis and reception. This research integrates physiology with the molecular mechanisms underlying insect metamorphosis.

Aims

1. Measure JHIII concentrations in hemolymph throughout metamorphosis in *M. rotundata*.
2. To quantify relative gene expression of genes regulating JHIII and 20-hydroxyecdysone (ecdysone) synthesis, degradation, and reception (see figure below).



Methods

1. Extract hemolymph and quantify by HPLC-MSMS

2. Extract mRNA and quantify by qPCR

JHIII Synthesis

- mt
- mfe

JHIII Degradation

- Jhe

JHIII Receptor

- met

20-e Synthesis

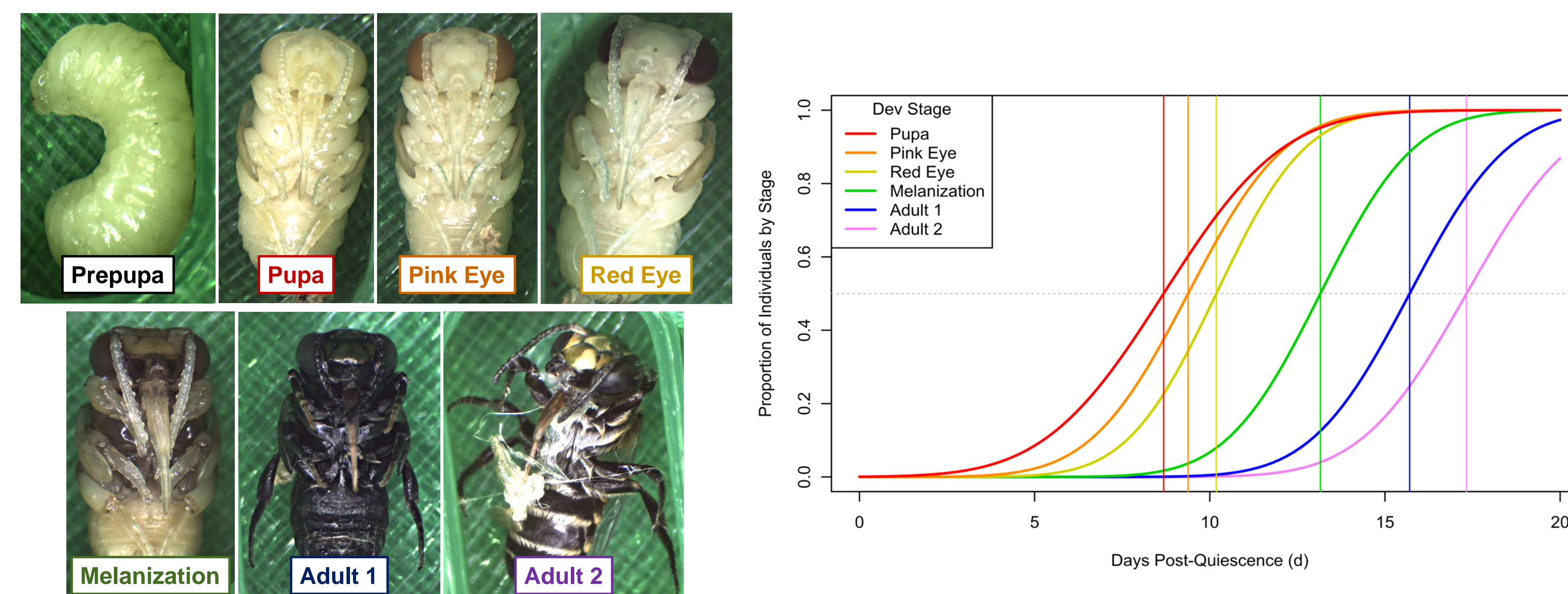
- shade
- shadow

20-e Receptor

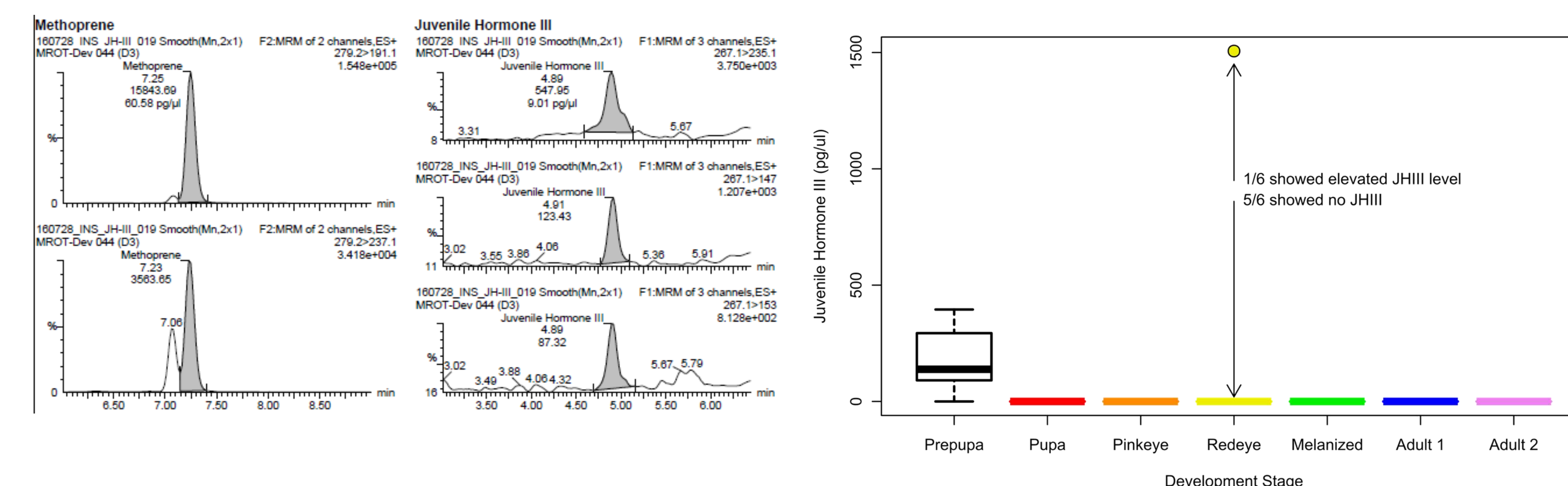
- EcR

Prepupa Pupa Adult

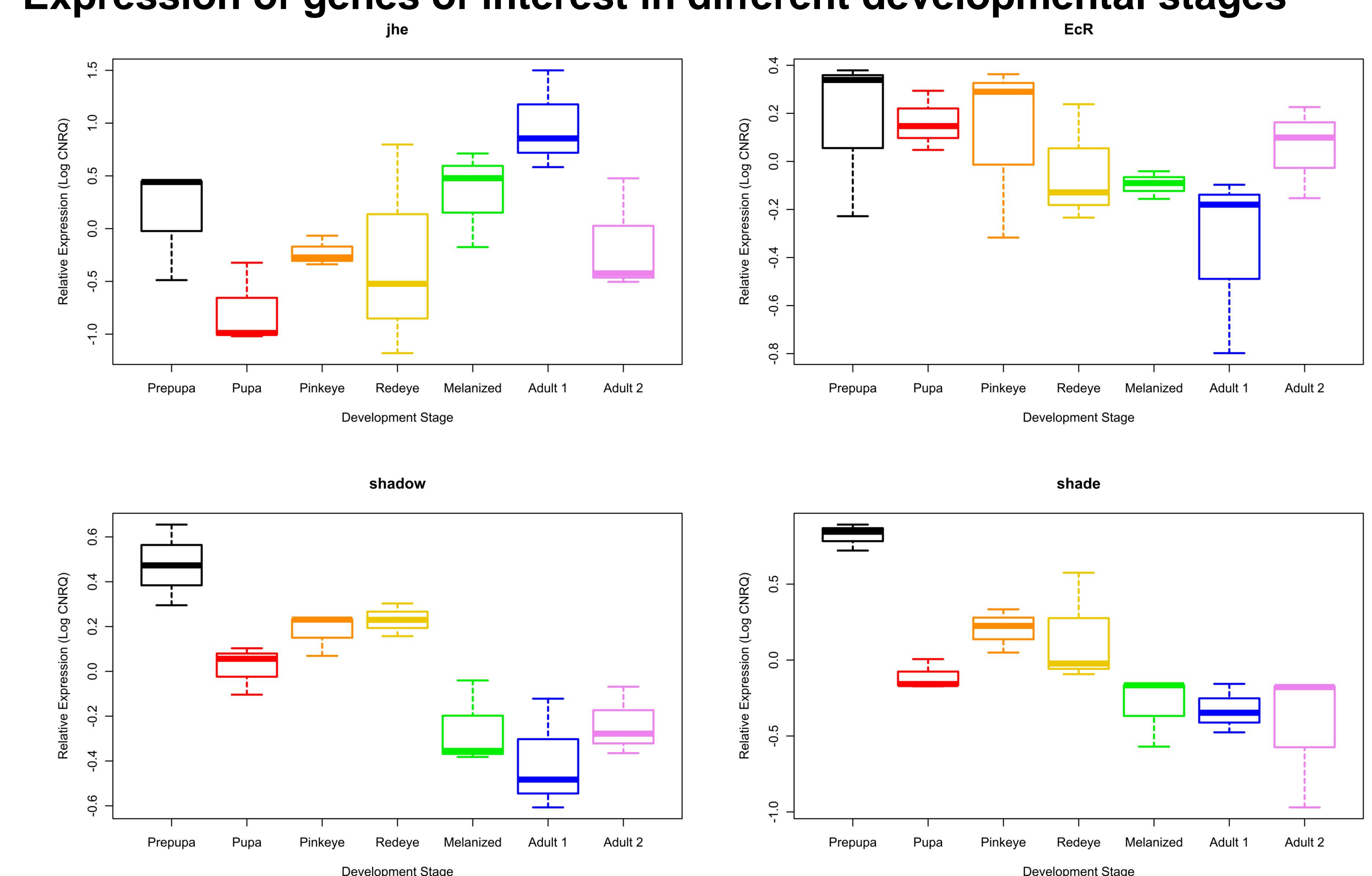
Timing of developmental stages during metamorphosis in *M. rotundata*



Quantification of hemolymph JHIII in different developmental stages



Expression of genes of interest in different developmental stages



Discussion Points

Developmental Timing Study

- There was high variability in life development stages. Better timing and staging synchronicity needed.

Juvenile Hormone Profiling

- JH was present in prepupa but not in latter stages with one outlier in the “red eye” stage. This hints that JH changes may occur on shorter time scales than our current sampling.

qPCR data and significance

- We successfully quantified expression of most of our target genes, including jhe, EcR, shade, and shadow. However, JH synthesis genes and receptor were not accurately quantified.

Acknowledgements

This work was supported by the USDA-ARS in Insect Genetics and Biochemistry, the Department of Biological Sciences at North Dakota State University, and NSF IOS 1557640 to JHB. We would also like to thank M. Larson and D. Melicher for helpful support in implementing the molecular protocols for this project.

References

1. Nijhout HF. Insect hormones. Princeton, NJ: Princeton University Press; 1994. 267 p.
2. Shingleton AW. Evolution and the regulation of growth and body size. In: Flatt T, Heyland A, editors. Mechanisms of Life History Evolution. 1st ed. New York: Oxford University Press; 2011. p. 43–55.
3. Gäde G, Hoffmann KH, Spring JH. Hormonal regulation in insects: facts, gaps, and future directions. *Physiol Rev.* 1997 77(4):963–1032.
4. Ares AM, Nozal MJ, Bernal JL, Martín-Hernández R, M. Higes, Bernal J. Liquid chromatography coupled to ion trap-tandem mass spectrometry to evaluate juvenile hormone III levels in bee hemolymph from *Nosema* spp. infected colonies. *J Chromatogr B.* Elsevier B.V.; 2012;899:146–53.

Affiliations

- a. Undergraduate, Department of Molecular and Microbiology, University of Central Florida, Orlando FL 32816
- b. Department of Biological Sciences, North Dakota State University, Fargo ND 58108
- c. USDA-ARS Animal Metabolism-Agricultural Chemicals Research, Fargo ND 58102
- d. USDA-ARS Insect Genetics and Biochemistry, Fargo ND 58102